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Effect of Ginger (*Zingiber officinale)* Rhizome Powder on Libido, Relative Organ Weight, Semen Quality and Histological changes in the Testes of Rabbit Bucks

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Abstract
The study was carried out to evaluate the effect of ginger on libido, relative organ weight, semen quality, and histological changes in the testes of rabbit bucks. Twenty – seven New Zealand White breeder rabbit bucks were assigned to 3 treatments (T_1 - T_3) of 3 replicates with 3 bucks per replicate in a CRD. Forage-based diet was supplemented with a formulated concentrate containing 17% CP and 2600Kcal/kg Metabolizable energy. 0, 10, and 20g ginger powder was added to 1kg of the concentrate and fed to T_1, T_2 and T_3 respectively daily. The concentrate was administered in the morning while forages were given in the afternoon through the night. Water was given *ad libitum.* Libido was measured while semen was collected and evaluated. The viscerals and testes were harvested and evaluated. The results showed significant (P<0.05) effect of treatment on all the semen quality parameters measured except semen pH, and normal spermatozoa. Semen volume was higher in T_3(1.55) and T_2(1.00) than T_1 (0.67). Semen consistency was highest in T_3(4.00) followed by T_2(3.00) and least in T_1 (2.00).T_2 and T_3 were statistically the same in sperm motility (83.33) and (83.00) and in percentage live spermatozoa proportion (86.90) and (88.70) and were higher than T_1 (76.70) and (79.46) respectively. Sperm concentration, total sperm /ejaculate, and total viable sperm increased with dosage and were better in T3 (131.55), (303.90) and (1593.30) respectively than T_2 and T_1. Spermatozoa morphology was not affected by the treatment (P > 0.05). Organ weights were not affected (P>0.05) except for the liver and testes which were significantly (P<0.05) higher in T_3 than T_2 and T_1. Libido score significantly (P<0.05) increased with dosage. Reaction time was significantly (P<0.05) lower and better in T_3 than T_2 and T_1. Testicular histology showed no structural morphologic abnormalities in the treated groups compared to the control. T_2 and T_3 had more accumulations of normal spermatids in the lumen of the seminiferous tubules. Mature spermatids significantly (P< 0.05) increased with dosage, 138/tubule in T_1, 148 and 152 spermatids per tubule in T_2 and T_3 respectively. It was concluded that supplementing the diet of breeder rabbit bucks with ginger powder at 10g/kg and 20g/kg levels will improve libido and semen quality without adverse effects on the viscerals and testes.

Keywords: Rabbit bucks, ginger, semen quality, histology

Introduction

Rabbits are micro livestock that have the potentials of supplying cheap and sustainable high animal protein to the ever-increasing human population in Nigeria (Onifade *et al*., *1999*). This is so because Nigeria is endowed with numerous grass lands that can supply enough forage for all – year – round rabbit production. Arijenuwa *et al*. (2000) reported that increased supply of rabbit meat is achievable because rabbits have short generation interval and can efficiently convert forage to edible animal protein. Thus, increased rabbit production, expressed as the number of kits kindled per doe per year and the survival of the kits (Lukefahr *et al*., 1991) is needed to put an end to animal protein shortage in Nigeria. However, intensive and large-scale rabbit production in hutches has been constrained by high kit and adult rabbit mortality. It appears that the use of high proportion of concentrate to meet the nutritional requirements of rabbits is associated with fatal bloat. Also, the use of some forage at certain seasons, physiological age and state could be responsible for many fatal nutritional disorders that are looming the rabbit industry in Nigeria. Consequently, there has been a quantum decline in production and collapse of many farms in Nigeria. Unfortunately, unlike avian, bovine and swine medicines rabbit medicine is less emphasized

in Nigeria. Thus, sudden death has continued to ravage many rabbit farms in Nigeria. In view of the foregoing, the need for complementary and alternative medicines that are readily available, safe, effective and economical (Baatsch *et al*., 2017) is imperative. Complementary and alternative medicines represent a group of diverse medical and healthcare systems, practices, and products that are not considered to be part of evidence–based conventional medicine (Yun *et al*., 2021). These complementary alternatives are of natural sources such as herbs and essential oils (Priya *et al.,* 2015) which have potential beyond clinical trials (ADA, 2019) and are rarely recommended to farmers. A typical example of such as herbal alternative is ginger. Empirical studies have shown that ginger has anti– oxidant, antimicrobial and anti–inflammatory properties which improve, semen quality parameters, sperm production and reproductive efficiency (Akhlaghi *et al*., 2014; Ezzat *et al*., 2017; Zhou *et al*., 2022; Al – Khalaifah *et al*., 2022). For instance, in roosters, it has been documented 15 and 30g/kg/day ginger supplemented diets significantly improved sperm motility, viability and fertility, and decreased structural abnormalities after 14days (Akhlaghi *et al*., 2014). Also, Saeid *et al*. (2011) and Ezzat *et al*. (2017) reported significant improvements in ejaculate volume, total sperm, serum LH, FSH and testosterone levels in roosters fed 5%, 10% and 2.5g/kg and 5g/kg ginger rhizome powder after 64weeks and 12weeks respectively. However, in rats, it was also documented that 2000mg/kg/bw/day of ginger showed gonadotoxic activity, caused follicular atresia,and degenerated primordial follicle, indicating that it has antifertility, antiimplatation and antiovulatio effect(Elmazoudy and Attia, 2018) Similarly, Herawati (2010) reported oedema, liver necrosis and inflammation in broiler chickens fed 0.1, 1.0 and 1.5% ginger powder. It was also observed that 500mg/kg ginger produced brady cardia with degenerative changes in the cardiac myocyte fibres of rats (Singh et al., 2018). In rabbits, it was reported that 5g/kg oral administration of ginger caused 50% mortality (Omayma *et al.,* 2018). There are controversies over the safety and toxicity of ginger with respect to dose, form (extract or powder) and the duration of administration. Thus, more scientific investigations in these regards have been advocated (Gagneir, 2006). It was observed that most researches on ginger were on roosters and albino rats, with scanty publications on rabbits. Also, research reports on the histological in the testes of rabbit buck fed ginger powder are scanty. In this study, doses reported to be safe and toxic were selected to evaluate their effects on semen quality parameters and testicular histopathology.

Materials and methods

Experimental location

The experiment was conducted at the Rabbit Unit of the Teaching and Research Farm of the College of Animal Science and Animal Production, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

Procurement of the test ingredient

Ginger rhizome powder was purchased from National Root Crops Research Institute, Umudike, Abia State, Nigeria. The ginger powder was stored in an airtight plastic container under room temperature.

Management of experimental animals

A total of twenty - seven (27) New Zealand White breeder rabbit bucks (10 months of age) were used for the experiment. The bucks were purchased from a reputable farm in Umuahia North L.G.A., Abia State. The rabbit bucks were raised in hutches and fed formulated diet in the morning (8am – 3pm) and forage in the evening (3p.m – 8am) daily. Fresh water was given *ad libitum.* The bucks were randomly divided into 3 treatments (T_1 – T_3) with 9 bucks per treatment and replicated 3 times with 3 bucks per replicate. T_1 received 0g/kg diet, while T_2 and T_3 received 10g/kg and 20g/kg of ginger supplemented diets respectively with plenty of forages. The composition of the diet is presented in Table 1.

Semen collection and evaluation

The rabbit bucks were trained for semen collection by introducing a teaser doe in the hutch for the bucks to sniff and mount without intromission and ejaculation Semen was collected with an artificial vagina and assembled as described by Herbert and Adejumo (1995). Ejaculate samples were collected and analyzed. Semen volume was measured with a calibrated plastic centrifuge tube and recorded in milliliter. Semen consistency was assessed and scaled according to Bearden *et al*. (2004). Semen pH was measured with a (ROTA MEB) pH meter. Libido was evaluated and scored according to Herbert and Adejumo (2005). Individual sperm motility was determined by placing a drop of fresh semen on a pre-warmed glass slide. The drop of semen was diluted with an equal volume of pre-warmed 0.9% normal saline and covered with a pre-warmed cover slip. The individual sperm motility was subjectively scored in percentage. Sperm concentration ($\times 10^9$ /ml) was determined using an improved haemocytometer after dilution with 0.9% physiological saline in the ratio of 1:200. Total sperm/ejaculate was determined by multiplying the semen (ejaculate) volume by the calculated sperm concentration. The proportion of live spermatozoa was evaluated using the method described by Bearden *et al*. (2004). A drop of fresh semen was placed on a pre-warmed glass slide and mixed with a drop of pre-warmed Eosin-nigrosin stain. A smear was made from the mixture and viewed under a high-power microscope (× l00 oil immersion objective). Total viable spermatozoa in ejaculate were determined using the relation: Volume (ml) × Conc. ($\times 10^9$ml) × % motility (%) × % Normal cells.

***Organ biometry/histology*:** At the end of the experiment, two rabbits were randomly selected from each treatment replicate and sacrificed. The organs were harvested and weighed with a sensitive scale and recorded in gram. Histology of the testes was carried out according to Clayden (1967) and John and Alan

(1977). Images were captured with a motican 2.0 digital camera attached to a computer.

Experimental design and statistical analysis

The experiment was arranged as a CRD. Data collected were subjected to analysis of variance (ANOVA) (Steel and Torrie, 1980). Means were separated using Duncan' multiple range test (Duncan, 1955). The linear statistical is as shown below:

Linear model:

$Y_{ij} = \mu + B_i + e_{ij}$

Where:

Y_{ij} = Individual observation
μ = Overall mean
B_i = Effect of treatment
e_{ij} = Random error.

Results and Discussion

The effect of ginger rhizome powder on semen quality parameters is shown in Table 2. The result of this study showed significant (P<0.05) effect of ginger treatment on the semen quality parameters measured except semen in pH and percentage normal spermatozoa. T_2 and T_3 were better than T1 in semen volume, consistency, sperm motility, live spermatozoa, sperm concentration, total sperm in ejaculate and total viable sperm. Semen pH, percentage normal and abnormal sperm were not affected by treatment (P > 0.05). The result of this study agreed with those of Akhlaghi *et al.* (2014) and El-Speiy *et al.* (2017), who reported higher ejaculate volume in roosters and rabbit bucks fed diets supplemented with ginger powder respectively. Semen volumes (1.0 – 1.55ml) obtained in this study were higher than (0.71ml, 0.93ml) and (0.3 - 0.61ml) reported by Herbert *et al.* (2005), Cardinali *et al.* (2007) and Lebas *et al*. (1997), respectively. This suggests that supplementing the diet of rabbit buck with ginger could significantly improve ejaculate volume. The reason for the higher semen volume obtained in this study could be a result of increased stimulation and secretory activities of the accessory sex glands (Shinkut, 2009) by the phenolic compounds in ginger. Semen pH was not affected by treatment (P > 0.05). This indicated that 10 and 20g/kg dietary inclusion of ginger did not induce alkaline and acid reaction in accessory glands that could lead to changes in pH. This is because changes in pH negatively influence spermatozoa viability and motility due to poor buffering capacity (Bearden *et al.,* 2004). The result obtained in this study showed that ginger has good buffering effect on rabbit buck semen. The result of this study showed significant (P < 0.05) effect of treatment on semen consistency. T_2 and T3 had better semen consistency than T_1. Viscosity improved as the inclusion levels increased. This is in consonance with Kaya *et al.* (2002), who reported that the opacity of semen provides a rough indication of concentration. Peter *et al.* (2005) reported a direct relationship between semen consistency and sperm concentration as thicker semen has greater number of spermatozoa than watery semen. This may imply that ginger powder supplemented at 10 and 20g/kg levels in the fed of rabbit bucks could significantly enhance the thickness of their semen.

The result of this study showed significant (P < 0.05) effect of treatment on spermatozoa progressive motility. Motility was higher in T_3 (85.00) and T_2 (83.33) than T_1 (76.70) respectively, suggesting that ginger supplementation could significantly enhance motility in rabbit bucks. The motility mean values obtained in this study for T_2 and T_3 were higher than (80%) normal range recommended by Bearden *et al*. (2004) for fertile ejaculate, indicating that ginger supplementation could improve fertility in rabbits. The result of this study was in agreement with those of Arash *et al*. (2009) and Ezzat *et al*. (2017) who reported significant increase in sperm motility and viability of Wister rats and roosters fed graded levels of ginger rhizome powder. The reason for the result obtained in this study could be due to antioxidative effect of ginger on the semen (Al Khalaifah *et al*., 2022) which decreases the malonaldehide and increases the total antioxidant capacity of semen (Majid *et al* 2021). The result of this study could also be attributed to the anti – inflammatory property of ginger which improved resistance against infectious agents and reproductive performance (Zhou *et al.,* 2022). The mean percentage live spermatozoa proportion followed a similar trend as the progressive motility. T_2 and T_3 were significantly higher than T_1. The reason for the higher number of live spermatozoa could be due to the effect of the ginger on the proliferation and fast maturation of developing sperm cells in the seminiferous tubules which perhaps increased both the supply of FSH and testosterone levels (Memudu *et al*., 2012; Saeid *et al*., 2011; Ezzat *et al*., 2017) as was observed in the testicular histology and libido evaluation in this study. The study further confirmed the direct relationship between libido and sperm concentration as opined by Herbert *et al.* (2005). The total number of spermatozoa per ejaculate and its corresponding total viable sperm cell was significantly (P<0.05) increased as the inclusions were increased compared with the control group. The mean total viable sperm cell which is the product of semen volume, concentration, percentage motility and normal spermatozoa, was significantly (P<0.05) increased from 521.77 x 10^9/cell (T1) to 948.20 x10^9/cell and 1593.30 x 10^9/cell in T_2 and T_3, respectively.

This implies that ginger at the inclusion levels used in this study did not cause any deleterious effect on the morphological indices of the spermatozoa. The result of semen characteristics obtained in this study strongly agreed with El-Speiy *et al.* (2017) and Ilo *et al. (*2018) who reported significant improvement in semen quality of bucks fed diets supplemented with ginger at 0.5% and 1.0%, respectively. The effect of treatment on spermatozoa morphology is shown in Table 3. The result of this study did not show any significant (P > 0.05) effect of ginger on spermatozoa morphological abnormalities. The ability of the sperm cells to maintain their normal morphology and integrity could be due to

the antioxidant effect of ginger in the formulated diets in T_2 and T_3. This could mean that while enhancing the semen quality, the ginger supplemented diet did not affect the morphology of the spermatozoa. The result of this study is in consonance with those of Khaki *et al.* (2009) who reported an increased percentage of normal spermatozoa in rats fed 50 and 100mg/kg ginger extract. The effect of ginger on the relative organ weight of rabbit bucks is shown in Table 4. The result of this study did not show significant ($P>0.05$) effect of the test ingredient on the weights of the heart, spleen, and kidney relative the body weights of the bucks. However, the liver and the testes weighed heavier ($P<0.05$) in T_2 and T_3 than T_1. The non-significant effect of the ginger diet on the liver, kidney and heart was in consonance with the report of Agunbiade *et al*. (2001), Tangpu and Yadav (2006), and Ilo *et al.* (2018). The paired testicular weight was higher in T_3 (0.42%) than in T_2 (0.37%) and least in T_1(0.23) The result of this study was in agreement with those of EL Speiy *et al.* (2017) also reported an increase in paired testicular weight of bucks fed a 10% ginger inclusion diet. Similarly, Arash *et al. (2009)* reported increased testicular weight, serum testosterone level, and accumulation of sperm in the lumen of the seminiferous tubules of Wister male rats administered with 50mg/kg and 100mg/kg ginger rhizome powder. These increases in the testicular ultrastructure and in the number of sperm cells could be the reason for the heavier testicular weights obtained for T_2 and T_3 in this study. The effect of ginger on libido score of rabbit bucks is shown in Table 5. The result of this study indicated significant ($P<0.05$) effect of the test ingredient on the libido score of the bucks. Libido was higher in T_2 and T_3 than T_1. Reaction time was also affected significantly ($P< 0.05$) by ginger supplementation. Reaction time was lower and better in T_3 than T_2 and T_1. This could be attributed to the androgenic effect of ginger (Amr and Hamza, 2006) which increased the serum testosterone level and improved libido of the bucks in a dose dependent manner.

Testicular histology of rabbit bucks fed diets supplemented with ginger powder

According to Hafez (1993) any stimulation of Leydig cells will result in increased testosterone production, which will in turn increase spermatogenesis and libido. The effects observed in this study at higher inclusion of ginger as shown in the photomicrograph, may be due to enhancement of the Leydig cells and seminiferous tubules responsible for libido and sperm productions respectively. Furthermore, the stimulation of meiosis of primary spermatocytes into secondary spermatocyctes and haploid spermatids (the meiotic cell division phase of spermatogenesis) and an increase in mitotic activity, resulted in the production of many mature spermatozoa from a density of 138 (T_1) to 152 in the highest inclusion group (T_3). The testicular histology of the ginger treated bucks appeared normal with intact spermatogenic cell lineage, containing all the cells in the lineage.

Conclusion

The result of this study indicated that 10g/kg and 20g/kg ginger rhizome powder improved semen quality parameters and libido with neither gross atrophy nor inflammation of the viscerals and testes. There were also no histological changes in the testes. It was concluded that breeder rabbit bucks can be fed with diets supplemented with up to 20g/kg ginger rhizome powder without any adverse effect on the reproductive performance. Based on the result of this study, 20g/kg ginger is therefore recommended.

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Table 1: Composition of the experimental diets

Ingredients	Composition
Maize	34.00
PKC	15.00
GNC	20.00
Maize offal	27.50
Salt	0.25
Bone meal	3.00
V/M premix	0.25
Total	**100.00**

Note: Calculated crude protein = 17%; Metabolized energy = 2600kcal/kg

Table 2*:Semen quality parameters of rabbit bucks fed diets supplemented with ginger rhizome powder*

Parameter	T_1	T_2	T_3	SEM
Semen volume (ml)	0.67^{b}	1.00^{ab}	1.55^{a}	0.09
Semen pH	6.81	6.78	6.74	0.02
(Scale: 1 – 14)				
Semen consistency (Score: 1-4)	2.00^{c}	3.00^{b}	4.00^{a}	0.24
Spermatozoa motility (%)	76.70^{b}	83.33^{a}	85.00^{a}	1.46
Spermatozoa live proportion (%)	79.46^{b}	86.90^{a}	88.70^{a}	1.76
Sperm cell concentration $\times 10^9$/cell)	110.34^{b}	123.59^{ab}	131.55^{a}	3.33
Total no. of sperm cell/ejaculate ($\times 10^9$/ml)	73.93^{c}	123.59^{b}	203.90^{a}	13.19
Total viable spermatozoa$\times 10^{12}$/ml)	521.77^{b}	948.20^{ab}	1593.30^{a}	88.13
Percentage normal sperm cell (%)	92.02	92.07	91.93	0.58

^{abc}Means with different superscripts in the same row are significantly(P<0.05) different. SEM: Standard Error of Mean

Table 3: Effect of *ginger* on spermatozoa morphology of rabbit bucks

Parameter	T_1	T_2	T_3	SEM
Headless spermatozoa (%)	0.76	1.37	1.46	0.18
Twisted tail spermatozoa (%)	3.67	3.22	3.19	0.31
Tailless spermatozoa (%)	2.13	2.56	3.03	0.24
Broken neck spermatozoa (%)	0.34	0.21	0.12	0.08
Cytoplasmic droplet (%)	1.06	0.57	0.27	0.27
Total abnormality (%)	7.98	7.93	8.09	0.57

Means without superscripts are not statistically (P> 0.05 significant. SEM: Standard Error of Mean

Table 4: The effect of effect of ginger on relative organ weights of rabbit bucks

Parameter	T1	T2	T3	SEM
Live weight (kg)	1.54	1.95	1.78	0.08
Liver (g)	2.92^{b}	3.01^{ab}	3.37^{a}	0.10
Heart (g)	0.21	0.19	0.20	0.01
Spleen (g)	0.02	0.02	0.03	0.00
Kidney (g)	0.45	0.51	0.52	0.02
Paired testes (g)	0.23^{c}	0.37^{b}	0.42^{a}	0.02

^{abc}Means with different superscripts in the same row are significantly(P<0.05) different. SEM: Standard Error of Mean

Table 5: Effect of ginger on libido score of rabbit bucks

Parameter (sec)	T_1	T_2	T_3	SEM
Libido	2.57^b	3.89^a	4.20^a	0.36
Reaction time	8.43^a	5.26^b	4.12^c	0.41

[abc]Means with different superscripts in the same row are significantly(P<0.05) different. SEM: Standard Error of Mean

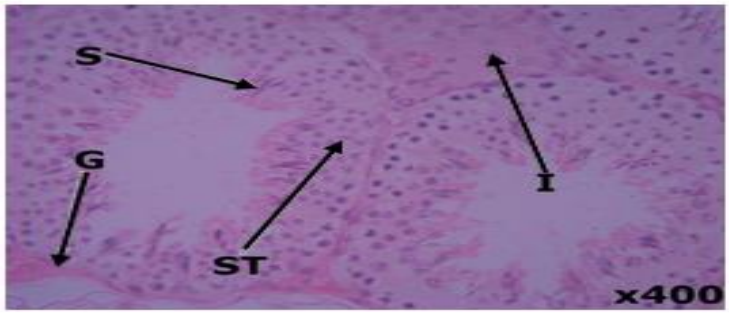

Plate 1: *Photomicrograph of testes shows intact seminiferous tubules and interstitium with orderly germ cell maturation variable around the tubule. The mature spermatid density on average was 138 per tubule (**T_1**);* ***Key:*** *G – Germinative layer; I=Interstitial cells of Leydig; ST=Sertoli cell; S=Mature Spermatid*

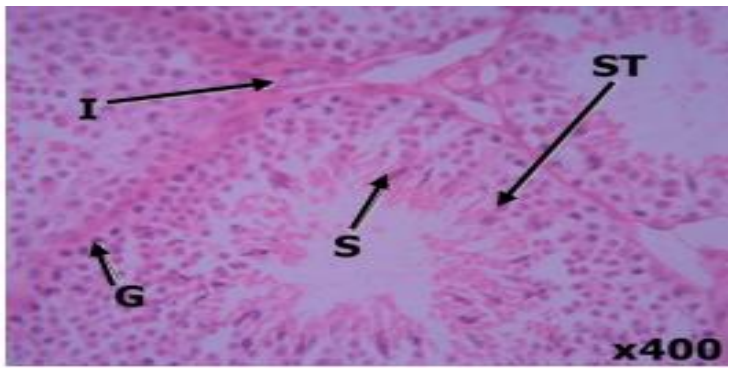

Plate 2: *Photomicrograph of testes shows intact seminiferous tubules and interstitium with orderly germ cell maturation variable around the tubule. Compared with control, the spermatid density of about 148 were counted per tubule in the rabbit buck fed with 10g/kg of feed (**T_2**).* ***Key:*** *G – Germinative layer; I=Interstitial cells of Leydig; ST=Sertoli cell; S=Mature Spermatid.*

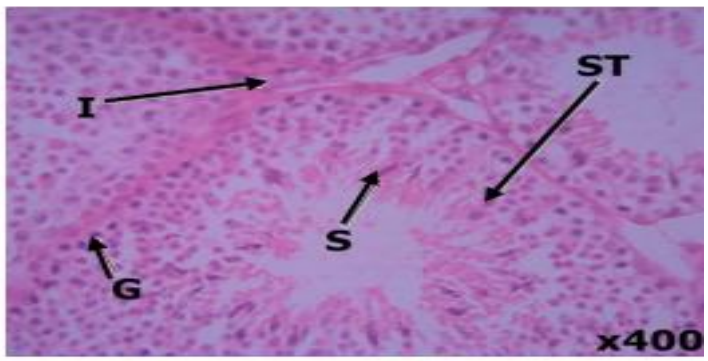

Plate 3: *Photomicrograph of testes shows intact seminiferous tubules and interstitium with orderly germ cell maturation variable around the tubule. Compared with control (T_1), there were about 152 spermatids per tubule in the rabbit buck fed with 20g/kg of feed (**T_3**).* ***Key:*** *G – Germinative layer; I=Interstitial cells of Leydig; ST=Sertoli cell; S=Mature Spermatid.*